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Estimation of the local production of antibodies to Treponema pallidum in the central nervous system of patients with neurosyphilis

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SUMMARY In 76% of 149 patients with neurosyphilis local production of treponema-specific IgG antibodies in the central nervous system (CNS) was determined by estimating the cerebrospinal fluid (CSF)-to-serum ratio of TPHA-IgG titre per mg total IgG. With this formula synthesis of treponema-specific IgG antibodies in the CNS could be detected independently of the function of the blood-CSF barrier. A selective increase of total IgG in the CSF was not found in all cases. Forty-three patients with adequately treated or spontaneously resolved syphilis without clinical evidence of involvement of the CNS by *Treponema pallidum* served as controls.

Introduction

The concentration of albumin, IgG, and other proteins in the cerebrospinal fluid (CSF) of healthy adults depends on their concentrations in the serum.[1] The serum-to-CSF ratio of albumin is usually about 220. This has proved to be a reliable indicator of the function of the blood-CSF barrier, since albumin is not synthesised in the central nervous system (CNS) but is derived entirely from serum. The serum-to-CSF ratio of IgG is about 450.[1-4]

In patients with inflammation of the CNS the transudation of serum proteins into the CSF is increased as a result of the increased permeability of the blood-CSF barrier; this reduces the serum-to-CSF ratio of all proteins. On the other hand T and B lymphocytes may infiltrate the CNS during acute infections and result in local synthesis of immunoglobulins.[5]

An increase in total IgG in the CSF of patients with neurosyphilis is thought to be the result of local production of antibodies to *Treponema pallidum*.[6 7] More specific evidence of the local synthesis of specific immunoglobulins can be gained by serological tests which show a reduced serum-to-CSF ratio of antibodies to *T pallidum*.

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The present study was undertaken to determine whether the production of treponema-specific antibodies in the CNS of patients with neurosyphilis is independent of the function of the blood-CSF barrier and the selective increase in total IgG in the CSF. For this purpose we measured the serum-to-CSF ratios of albumin, total IgG, and treponema-specific IgG as well as the CSF-to-serum ratio of treponemal IgG per mg of total IgG in patients with syphilis both with or without clinical features of neurosyphilis.

Materials and methods

SERUM AND CEREBROSPINAL FLUID SAMPLES

Paired samples of serum and CSF were collected on the same day from 212 patients with several different clinical disorders attending neurological clinics. A diagnosis of syphilis was made when the *T pallidum* haemagglutination assay (TPHA) and the fluorescent treponemal antibody-absorption (FTA-ABS) test on the patients' sera gave positive results. In each case a detailed history, including that of specific treatment, was obtained.

QUANTITATIVE DETERMINATION OF ALBUMIN AND TOTAL IgG

Quantification of albumin and total IgG in serum and CSF samples was carried out by single radial immunodiffusion using Partigen plates (Behringwerke, Marburg, Germany).

DETERMINATION OF TREPONEMA-SPECIFIC IgG TITRE/MG TOTAL IgG

In detecting antibodies against pathogenic treponemes the specificity of the TPHA was higher than 99·9%.[8] The specificity of the test on CSF samples was as high as that on sera. The test was carried out quantitatively according to the manufacturer's instructions (Fujizoki Pharmaceuticals, Tokyo, Japan). Total TPHA titres were estimated by the dilution of 25 µl serum or CSF in absorbing diluent at a starting dilution of 1/5 (serum) or 1/1 (CSF). If treponemal IgM antibodies were found in the sample the TPHA-IgG titre was calculated by subtracting the TPHA-IgM titre (see below) from the total TPHA titre.

The specific TPHA-IgG titre per mg total IgG was calculated from the TPHA-IgG titre in the serum or CSF divided by the total IgG in mg/25 µl.

FRACTIONATION OF SERA AND CSF BY GEL FILTRATION

Separation of IgM from IgG antibodies in the sera or CSF was carried out by Ultrogel AcA 34 filtration according to the method of Müller and Oelerich.[9] Briefly, 0·7 ml of the sample was filtered through a 1·5 × 14 cm gel column using phosphate-buffered saline, pH 7·3. Fractions of 1·3 ml were collected and the absorbance at 280 nm measured.

19S(IgM)-TPHA TEST

The method used for this test was that described by Müller and Lindenschmidt.[10] Fractions from the first peak absorbance (IgM) after gel filtration of serum or CSF were titrated (starting dilution 1/1) using the sensitised erythrocytes of the TPHA test as antigen. The titres obtained were multiplied by 6 (the dilution factor of the gel filtrate). The specificity of the 19S(IgM) treponema-specific antibodies was shown by the 19S(IgM)-FTA-ABS technique.

19S(IgM)-FTA-ABS TEST

The technique for demonstrating treponema-specific 19S(IgM) antibodies in the fractions after gel filtration has been described.[9-11] The Nichols strain of pathogenic *T pallidum* was used as antigen. For the detection of 19S(IgM) treponema-specific antibodies FITC-labelled anti-human IgM serum from rabbits with µ-chain specificity (Dako Immunochemicals, Copenhagen, Denmark) was used at a working dilution of 1/50.

Results

The patients were divided into nine groups on the basis of the serum-to-CSF ratios of albumin, total IgG, TPHA-IgG titre, and the CSF-to-serum ratio of TPHA-IgG titre per mg total IgG.

Group 1a—In 43 patients syphilis had been treated satisfactorily or had resolved spontaneously. From the mean concentrations, standard deviations (2 SD), and ranges for albumin and total IgG concentrations in the sera and CSF it can be seen that neither the function of the blood-CSF barrier nor the CNS was affected by the treponemal infection (table I). Treponema-specific IgM antibodies could not be detected in the serum or in the CSF of these patients. Correspondingly, the serum-to-CSF ratios of albumin and total IgG were within the normal range (table II). The concentrations of TPHA-IgG antibodies in 1 mg total IgG were equal in both serum and CSF. The mean value of the CSF-to-serum ratio was 1.

Group 1b—Normal values were found in 13 patients with untreated secondary syphilis who had no clinical or biochemical evidence of CNS involvement (table II). Sera from all of these patients were reactive in the 19S(IgM)-FTA-ABS test.

Group 1c—Normal values were found in 10 patients with adequately treated neurosyphilis but residual symptoms of tabes dorsalis or general paralysis of the insane.

Groups 2a and 2b—Of 81 patients with neurosyphilis with normal serum-to-CSF albumin ratios, 48 had normal serum-to-CSF ratios of total IgG but reduced ratios of TPHA-IgG and increased CSF-to-serum ratios of TPHA-IgG titre per mg total IgG. In 33 of the 81 patients with neurosyphilis an increase of total IgG as well as of TPHA-IgG in the CSF was shown by reduced serum-to-CSF ratios. The CSF-to-serum ratio of TPHA-IgG titre per mg total IgG was raised appreciably in each patient. The TPHA-IgG titres per mg of total IgG in the serum

TABLE I *Mean values, standard deviation (2 SD), and range of concentrations of albumin and IgG in the sera and CSF of patients whose infection with syphilis had been treated or had resolved spontaneously without involvement of the CNS*

	Albumin (mg/l)			*Total IgG (mg/l)*		
	Mean	*2 SD*	*Range*	*Mean*	*2 SD*	*Range*
Serum	39 900	15 500	24 600-54 900	11 500	5200	7100-18 600
CSF	198	120	102-304	29	20	13-47

TABLE II *Findings in 149 patients with neurosyphilis, 20 patients without neurosyphilis, and 43 controls*

Group*	No of patients	Serum-to-CSF ratio of: Albumin Mean	Albumin Range	IgG Mean	IgG Range	TPHA-IgG titre Mean	TPHA-IgG titre Range	CSF-to-serum ratio of: TPHA-IgG titre/mg IgG Mean	TPHA-IgG titre/mg IgG Range
1a	43	220	143-392	446	268-851	445	320-640	1·0	0·5-2·0
1b	13	226	149-366	460	316-485	440	320-640	0·9	0·5-1·9
1c	10	208	159-268	378	273-568	378	320-512	1·0	0·6-1·8
2a	48	230	143-397	441	268-742	48	0·6-160	41	3·0-430
2b	33	203	146-390	158	36-257	18	0·2-80	31	3·0-178
3a	14	84	9-122	130	17-255	89	10-160	1·4	0·8-2·0
3b	34	109	31-139	145	10-263	20	1-80	19	3·0-139
4a	10	110	80-126	226	174-261	320	317-320	0·7	0·6-1·0
4b	7	96	26-135	165	32-267	494	320-640	0·3	0·1-0·4

*Group 1a—adequately treated or spontaneously resolved syphilis without symptoms of neurosyphilis; group 1b—untreated secondary syphilis without symptoms of neurosyphilis; group 1c—adequately treated neurosyphilis with characteristic symptoms; group 2a—neurosyphilis with intact blood-CSF barrier and normal total IgG in the CSF; group 2b—neurosyphilis with intact blood-CSF barrier, selective increase of total IgG in the CSF, and local synthesis of treponema-specific IgG antibodies; group 3a—neurosyphilis with dysfunction of the blood-CSF barrier without local synthesis of treponema-specific IgG antibodies; group 3b—neurosyphilis with dysfunction of the blood-CSF barrier with local synthesis of treponema-specific IgG antibodies; group 4a—neurosyphilis without local synthesis of treponema-specific IgG antibodies; group 4b—syphilis with synthesis of treponema-non-specific IgG in the CNS.

and CSF of patients with neurosyphilis (groups 2a and 2b) are shown in fig 1. In both groups the corresponding values were up to 2·6 $\log_{10}$ higher in the CSF than in the serum.

Groups 3a and 3b—Patients in these two groups with neurosyphilis had a reduced serum-to-CSF albumin ratio. In the 14 patients in group 3a the TPHA-IgG titres per mg total IgG were identical in both serum and CSF. This ratio was, however, increased in the 34 patients in group 3b (table II).

Groups 4a and 4b—The blood-CSF barrier of patients with syphilis was also disturbed (table II). In each patient in group 4a the ratio of treponema-specific IgG antibody was normal but the total IgG in the CSF was increased. A non-specific increase of total IgG in the CSF was found in the patients in group 4b. The CSF-to-serum ratio of TPHA-IgG titre per mg total IgG was below the lowest normal value of 0·5.

The TPHA-IgG titres per mg total IgG in the serum of the patients with dysfunction of the blood-CSF barrier are plotted against those in the CSF in fig 2. The patients of group 3b with higher values in the CSF than in the serum are located above the dotted lines (normal range). The patients in groups 3a and 4a (values within the dotted lines) had similar CSF and serum TPHA-IgG titres per mg total IgG. Below the normal range (group 4b) the TPHA-IgG titres per mg total IgG were lower in the CSF than in the serum.

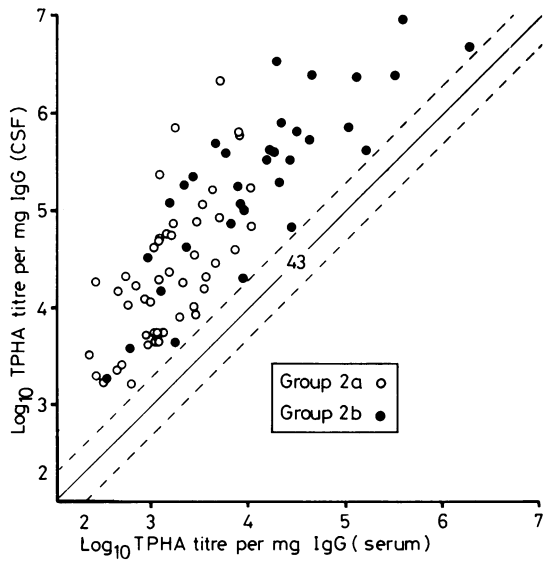

FIG 1 *Correlation of the TPHA-IgG titre per mg total IgG in CSF and serum of patients without dysfunction of the blood-CSF barrier. The dotted lines indicate the normal range of correlation (group 1a).*

Discussion

At present involvement of the CNS in syphilis is diagnosed by reactive TPHA or FTA-ABS test results on the CSF of patients with clinical features of the disease, white cell count in the CSF, and the estimation of the serum-to-CSF ratios of albumin and total IgG. Since the introduction of the FTA-ABS test and the TPHA treponema-specific IgG antibodies can be estimated quantitatively in serum and CSF.

The specificity of TPHA-IgG titres in the CSF is as high as in the serum. This can be shown by the

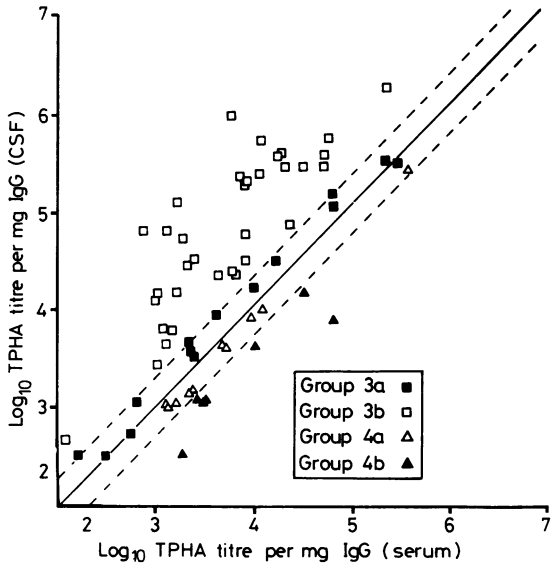

FIG 2 *Correlation of the TPHA-IgG titre per mg total IgG in CSF and serum of patients with dysfunction of the blood-CSF barrier. The dotted lines indicate the normal range of correlation (group 1a).*

finding of a normal serum-to-CSF ratio of TPHA-IgG titre in the groups of patients with syphilis but without involvement of the CNS. Parallel investigations in several patients using the IgG-FTA-ABS test instead of the TPHA-IgG test gave identical results. For this study we chose the TPHA because of its higher sensitivity to treponemal antibodies.

The following formula:

$$\frac{\text{TPHA-IgG titre per mg total IgG (CSF)}}{\text{TPHA-IgG titre per mg total IgG (serum)}}$$

was used firstly in patients with syphilis without clinical and biochemical signs of CNS involvement, that is with a normal white cell count and without local synthesis of total IgG in the CSF or dysfunction of the blood-CSF barrier (group 1a). The serum-to-CSF ratio of treponema-specific IgG per mg total IgG was 1 (range 0·5-2·0). This means that in these patients the concentrations of TPHA-IgG antibodies per mg total IgG were equal in serum and CSF. Furthermore, the serum-to-CSF ratio of TPHA-IgG titre correlated with the total IgG as well as with the values of normally occurring IgG antibodies.[12] These observations indicate that local synthesis of treponema-specific IgG does not take place in the CNS and that the treponemal IgG antibodies in the CSF are derived solely from the serum by transfer through the intact blood-CSF barrier.

Using the above formula local production of treponema-specific IgG can be shown in patients with a normal cell count and normal concentration of total IgG in the CSF, indicating involvement of the CNS in the treponemal infection (group 2a). In the patients in group 2b an increase in total IgG in the CSF was related to the synthesis of treponema-specific IgG in the CNS.

In the 14 patients in group 3a the reduced serum-to-CSF ratio of TPHA-IgG titre was caused by an increased inflow of treponema-specific IgG antibodies from the serum into the CSF as a result of dysfunction of the blood-CSF barrier. This was shown by identical TPHA-IgG titres per mg total IgG in both serum and CSF. Local synthesis of treponemal IgG antibodies can also occur in patients with a breakdown of the blood-CSF barrier as shown by the increased CSF-to-serum ratio of TPHA-IgG titres per mg total IgG in the 34 patients in group 3b.

From the results of the patients with neurosyphilis local production of treponema-specific IgG in the CNS occurred in more than 76% of cases. The formula shows that non-specific local production of IgG occurs in the CNS of patients with syphilis with neurological symptoms independent of the treponemal infection (group 4b). This might be explained by an inflammation of the CNS which is independent of the treponemal infection.

The boundaries between groups 2a and 2b as well as between groups 3a and 3b were fluid (figs 1 and 2). This is not surprising since in each patient time elapses between infection and healing and the numbers of treponema-specific IgG-producing B lymphocytes diminish at different rates from the CNS.

In the patients in this study no correlation could be seen between local production of treponema-specific IgG antibodies in the CNS and clinically characteristic symptoms of neurosyphilis (tabes dorsalis, paralysis, meningovascular neurosyphilis). About one-fourth of the patients in whom synthesis of treponemal IgG antibodies in the CNS occurred had no apparent neurological symptoms (clinically asymptomatic syphilis) (unpublished data).

Our findings confirm those of Schliep and Felgenhauer,[7] who noted raised titres of treponema-specific antibodies in the CSF of patients with neurosyphilis and assumed that local synthesis of these antibodies had taken place in the CNS.

It is noteworthy that there was a parallel between the local production of treponema-specific antibodies in the CNS and the appearance of oligoclonal IgG bands in the CSF. In about 75% of the patients with neurosyphilis oligoclonal IgG bands could be seen in the CSF (unpublished data). This agrees with the findings of Vartdal *et al*[13] and Strandberg

Pedersen *et al*,[14] who found intrathecal synthesis of oligoclonal IgG antibodies with treponemal specificity in patients with untreated neurosyphilis.

The above formula can be modified and used to demonstrate local synthesis of treponema-specific IgM antibodies in the CNS in patients with inflammation of the CNS in latent or late latent treponemal infections.

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